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Doran R. Pace, Patent Attorney

AMENDMENT UNDER 37 CFR § 1.1
Examination Group 1655
Patent Application
Docket No. GJE-35
Serial No. 09/463,549

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner : Arun Chakrabarti, Ph.D.
Art Unit : 1655
Applicant : Daniel Henry Densham
Serial No. : 09/463,549
Filed : January 27, 2000
Conf. No. : 6468
For : Nucleic Acid Sequence Analysis

Box CPA
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

A Petition and Fee for a two-month Extension of Time through and including July 1, 2002, accompanies this Preliminary Amendment.

It is respectfully requested that the above-identified patent application be amended as follows:

In the Claims

Please substitute the following claim:

Claim 1 (twice amended):

- C1
1. A method for sequencing a polynucleotide, comprising the steps of:
 - (i) reacting a target polynucleotide with a polymerase enzyme immobilised on a solid support, and complementary nucleotides, under conditions sufficient for the polymerase reaction; and

(ii) detecting an effect consequent on the incorporation of a specific nucleotide complementary to the target polynucleotide into a nascent polynucleotide being synthesized as a result of the polymerase reaction, to thereby determine the sequence of the target polynucleotide, the detection being carried out by measuring a change in, or absorption of, radiation that occurs if the nucleotide is incorporated.

Please add the following new claims 36 and 37:

36. The method according to claim 1, wherein the complementary nucleotides are not labeled.

37. The method according to claim 1, wherein the effect detected results from a conformation or mass change of the polymerase that occurs upon incorporation of the nucleotide.

Remarks

Claims 1, 3-21 and 30-34 are pending in the subject application. By this Amendment, Applicant has amended claim 1 and added new claims 36 and 37. Support for the new claims and the amendments to claim 1 can be found throughout the subject specification, including, for example, at page 2, lines 25-29, and at page 3, line 36 through to page 4, line 5. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1, 3-21, 30-34, 36, and 37 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

The remarks that follow are directed to the rejections set forth in the Office Action dated January 29, 2002.

Applicant gratefully acknowledges the Examiner's withdrawal of the previous rejections under 35 USC §112 and 35 USC §102(b). Although the Examiner indicates in the Office Action that the previous rejections under 35 USC §112, first paragraph, have been withdrawn, Applicant assumes that the Examiner intended to indicate that the rejections under 35 USC §112, second paragraph, have been withdrawn. However, if this is not what the Examiner intended, Applicant respectfully requests clarification.

Claims 1, 3-9, 21, and 30-34 are rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854). In addition, all the pending claims are rejected under 35 USC §103(a) as obvious over Tsien *et al.* in view of Holzrichter *et al.* further in view of Schwarz *et al.* (1991), and in view of Chang *et al.* (U.S. Patent No. 5,801,042), and in view of O'Donnell (U.S. Patent No. 6,221,642), and in view of Rosenthal *et al.* (WO 93/21340), and in view of Vind (U.S. Patent No. 6,159,687), and in view of Smith *et al.* (U.S. Patent No. 5,753,439). Applicant respectfully traverses each of these grounds of rejection.

The Tsien *et al.* reference is cited as the primary reference in each of the above rejections. However, as the Examiner acknowledges, the Tsien *et al.* reference does not teach or suggest the use of an immobilized polymerase. The Examiner relies on the Holzrichter *et al.* patent as teaching the use of an immobilized polymerase. The Examiner asserts that the Schwarz *et al.* (1991) reference teaches the detection of nucleic acid incorporation by surface plasmon resonance signal over time in the infra-red spectrum. The Examiner relies upon the Chang *et al.* patent for teaching a competitive

inhibitor of the polymerase enzyme and the O'Donnell patent for teaching the beta-2 dimer complex of the *E. coli* DNA polymerase III with a target polynucleotide. The Examiner further asserts that the Rosenthal *et al.* reference teaches the Taq polymerase and that the Vind patent teaches reverse transcriptase as a polymerase. The Examiner cites the Smith *et al.* patent as teaching the detection of nucleotides by NMR using electromagnetic radiation.

Applicant respectfully asserts that the subject invention is not obvious in view of the cited references. None of the cited references, taken alone or in combination, teach or suggest the claimed invention. Although the Tsien *et al.* reference teaches a method for sequencing nucleic acids, the Tsien *et al.* reference only teaches the use of nucleotides that are "labeled" in some manner so as to be detectable in the nucleic acid strand being synthesized. However, Applicant's claimed methods can be used with nucleotides that are not detectably labeled. Applicant notes that the presence of a label on a nucleotide can hinder incorporation of the nucleotide into the complementary strand being synthesized. Rather than detection of a labeled nucleotide, the subject invention relies on detection, via radiation, of a conformational and/or mass change of the polymerase when a nucleotide complementary to the target polynucleotide is incorporated into the polynucleotide chain being synthesized by the polymerase.

The Holzrichter *et al.* patent relates to a general method for identifying biochemical and chemical reactions. The method described in the Holzrichter *et al.* patent relies on the use of a scanning probe positioned on or near a reaction site, which can then detect acoustic signals emitted directly from the reaction site or transmitted through a surrounding medium. The method measures vibrations created by local mechanical events. Applicant acknowledges that the Holzrichter *et al.* patent describes an immobilized polymerase. The requirement for an immobilized polymerase is only so that the probe can be positioned as close as possible to a fixed reaction site so that the acoustic/vibration measurements can be taken. Thus, in contrast to the Applicant's claimed method, which detects changes in, or absorption of, radiation from the polymerase/nucleotide/DNA reaction complex when nucleotide incorporation occurs, the Holzrichter *et al.* method detects acoustic signals or motions (vibrations) that result from a chemical reaction.

Applicant respectfully asserts that there is no motivation to modify the methods of Tsien *et al.* to include an immobilized polymerase because there is no teaching or suggestion that

immobilizing the polymerase in Tsien *et al.* method would provide better results than using a polymerase that is not immobilized. As noted above, the Tsien *et al.* reference relies on the detection of fluorescent molecules attached to nucleotides. In the Tsien *et al.* method, fluorescently labeled nucleotides are incorporated into the complementary strand of a template DNA molecule that is immobilized on a solid substrate. Detection of the incorporated nucleotide within a polynucleotide template does not require the positioning of a detector in close proximity to the DNA/polymerase reaction, and there is no teaching or suggestion that using template DNA immobilized on a substrate results in loss of sensitivity in the detection of fluorescence or that using a polymerase immobilized on a substrate would increase sensitivity of the detection method. Moreover, in the method of Tsien *et al.*, the detection of the incorporated nucleotide is not even dependent on the polymerase being present at that point; in fact, the polymerase and other components are washed away from the immobilized template DNA (see, for example, page 12, lines 29-34, and page 13, lines 24-28, of the Tsien *et al.* reference). Accordingly, Applicant respectfully asserts that at the time of Applicant's invention there was no motivation to modify the detection technique used in the Tsien *et al.* reference to incorporate an immobilized polymerase. Nor would there have been any motivation to modify the teachings of the Holzrichter *et al.* patent since the Holzrichter *et al.* method relies on detection of acoustic signals rather than labeled nucleotides.

Applicant's claimed invention does not rely on detection of acoustic signals (as in the Holzrichter *et al.* patent) or the detection of an incorporated nucleotide within a polynucleotide via fluorescence (as in the Tsien *et al.* reference), but instead is based on Applicant's surprising discovery that the incorporation of a nucleotide into the reaction site of a polymerase during synthesis of a DNA strand complementary to the target DNA sequence results in a conformation and/or mass change in the polymerase that can be detected using radiation measurements. Accordingly, Applicant respectfully asserts that cited references do not teach or suggest the claimed invention and, therefore, the claimed invention is not obvious. Reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

It should be understood that these amendments have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



Doran R. Pace
Patent Attorney
Registration No. 38,261
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: 2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

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Attachment: Marked-Up Version of Amended Claim